

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 5, line 12, with the following amended paragraph:

Fig. 2. A). The cDNA (SEQ ID NO:20) and putative amino acid (SEQ ID NO:1) sequences of δ II tryptase (~~SEQ ID NO:1~~). The δ II cDNA sequence matched the putative exon sequence of the mMCP-7-like II gene. The δ I cDNA (not shown in Fig. 2) matched the exonic sequence of the partial mMCP-7-like I gene. Consistent with the published gene sequences, there were two nucleotide differences between the two cDNA sequences; G²¹⁶ (δ II cDNA) to A (δ I cDNA) (nucleotide numbering starts from the translation initiation codon), and G²²⁶ (δ II cDNA) to A (δ I cDNA). Only the second of these differences results in an amino substitution (Val in δ II to Met in δ I). Actual nucleotide sequence of cloned RT-PCR product is shown in bold lower case lettering. The location of the forward (NF1) and reverse (NR1) primers are indicated by arrows. The first amino acid of the mature enzyme is italicized and in bold. The three members of the catalytic triad, His, Asp and Ser, are in capitals and underlined. Nucleotide numbering begins from the translation initiation codon (Met). Amino acid numbering begins from the first residue of the mature enzyme (Ile). The position of the forward ($\bullet \rightarrow$) and reverse ($\leftarrow \blacklozenge$) primers, and the Taqman probe (\equiv) for RTQ-RTPCR are indicated. **B).** The amino acid sequence of a portion of a variant δ tryptase polypeptide (SEQ ID NO:21). This variant is the product of alternative splicing which results in the excision of 27 nucleotides from the beginning of exon 4, and thus the deletion of 9 amino acids from the polypeptide when compared to the full length δ tryptase polypeptide. The location of the 9 amino acids present in the full length polypeptide but missing in the variant polypeptide is indicated by a vertical arrow (\downarrow).

Please replace the paragraph beginning on page 5, line 29, with the following amended paragraph:

Fig. 3. Amino acid sequences of δ I tryptase (SEQ ID NO:2) and δ II tryptase (SEQ ID NO:1) compared to that of tryptases α I (SEQ ID NO:22), α II (SEQ ID NO:23), β I (SEQ ID NO:24), β II (SEQ ID NO:25), and β III (SEQ ID NO:26). A dash (-) indicates the presence of an identical amino acid. Numbering begins at the first residue of the mature enzyme, which is indicated by an arrow (\blacktriangledown). The seven loops comprising the substrate binding cleft are boxed and

labelled A, B, C, D, 1, 2, and 3. The H, D and S of the catalytic triad are marked with a hash (#). The premature termination codons of the δ tryptases are marked with an X. The peptide sequence used as the immunogen for anti δ tryptase is underlined (.....).

Please replace the paragraph beginning on page 20, line 21, with the following amended paragraph:

PCR amplification of first strand cDNA was performed within 2h of the reverse transcription reaction. Initially a nested PCR approach was used to amplify cDNAs, using primers designed according to the sequence of a gene that we isolated independently and named delta (δ) tryptase (data not shown), and according to the published sequence of the mMCP-7-like genes (GenBank accession numbers AF099147 and AF098327) (Pallaoro *et al.*, 1999). Two sets of primers (F1 = 5'-CCC GTC CTG GCG AGC CCG-3' (SEQ ID NO:4)/R1 = 5'-CAG TGA CCC AGG TGG ACA C-3' (SEQ ID NO:5) and F2 = 5'-AGT GGC CAG GAT GCT GAG C-3' (SEQ ID NO:6)/R2 = 5'-TTT GGA CAG GAG GGG CTG GCT-3' (SEQ ID NO:7) were employed to amplify the initial product, and a single nested primer pair (NF1 = 5'-GAG CAA GTG GCC CTG GCA-3' (SEQ ID NO:8)/NR1 = 5'-GGA CAT AGT GGT GGA TCC AG-3' (SEQ ID NO:9), see Fig. 2A) was used on the resulting template. In later experiments a single primer pair (F3 = 5'-TGC AGC AAA CGG GCA TTG TTG-3' (SEQ ID NO:10), and R3 = 5'-AAA GCT GTG GCC CGT ATG GAG-3' (SEQ ID NO:11) was used to amplify δ tryptase cDNAs.

Please replace the paragraph beginning on page 22, line 17, with the following amended paragraph:

Oligonucleotide primers (forward primer DF1 = GGC CAC AGC TTT CAA ATC GT (SEQ ID NO:12), reverse primer DR1 = GCA GTT AGG TGC CAT TCA CCT T) (SEQ ID NO:13) and a Taqman probe DTP1 (6FAM-CCT GCC AGG GTG ACT CCG GAG GG) (SEQ ID NO:14) were designed using the PrimerExpress software (PE Applied Biosystems) to specifically detect reverse transcribed δ tryptase mRNA, and not the mRNA of other tryptases (see Fig. 2A). Co-amplification of genomic DNA was avoided by locating the forward and

reverse primers in separate exons and designing the probe so that it straddled the exon5/exon6 boundary.

Please replace the paragraph beginning on page 23, line 26, with the following amended paragraph:

NZ white rabbits (Institute of Medical and Veterinary Science, Gilles Plains, SA, Australia) were immunised with a δ tryptase-specific peptide that possessed an amino terminal cysteine and the δ tryptase residues Y¹⁶²HTGLHTGHSFQIVRDD¹⁷⁸ (SEQ ID NO:15) conjugated to diphtheria toxin (Mimotopes, Melbourne Australia). The peptide sequence, located in the region translated from exon 5, has, only ~50% identify to the α/β tryptases (see Fig. 3). A search of protein databases detected no other protein that shared this epitope.

Please replace the paragraph beginning on page 25, line 20, with the following amended paragraph:

The recombinant fusion protein included an N terminal His-patch thioredoxin region (to increase translation efficiency and solubility), an enterokinase (EK) recognition site (to allow activation of the pro-enzyme), the mature delta tryptase sequence, and C-terminal V5 and 6xHis tags (to aid detection and purification). As the δ tryptase cDNA (Genbank Accession AF206664) used to generate the expression construct did not include the sequence coding for the beginning of the mature tryptase, the forward primer (5' CAC CAT GAT TGT TGG GGG GCA GGA GGC CCC CAG GAG CAA GTG GCC CTG G 3' set forth as SEQ ID NO:16) was designed to include this region. A reverse primer (5'GGT GCC ATT CAC CTT GCA 3' set forth as SEQ ID NO:17) was designed immediately 5' of the stop codon. The resulting PCR fragment was directionally cloned into the pET102D-TOPO vector (Invitrogen), sequenced in both directions, and the construct used to transform BL21 DE3 cells. Following the addition of isopropyl-beta-thiogalactopyranoside (IPTG) (0.5mM final concentration), the bacterial cells were incubated for 6 hours at 37°C while being agitated vigorously. The cells were pelleted by centrifugation and resuspended in lysis buffer (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole pH 8.0). The lysate was centrifuged to remove cellular debris, and the His-tagged recombinant protein purified from the supernatant using a Ni-NTA column.

Please replace the paragraph beginning on page 27, line 25, with the following amended paragraph:

PCR amplification of first strand cDNA was performed within 2h of the reverse transcription reaction using the primers F3 = 5'-TGC AGC AAA CGG GCA TTG TTG-3' (SEQ ID NO:18), and R3 = 5'-AAA GCT GTG GCC CGT ATG GAG-3' (SEQ ID NO:19).

Please insert the attached Sequence Listing on a new page after the abstract.